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(54) Electrochemical sensors in immunological measurement.

(57) An electrochemical sensor system used for determining the quantity of glucose by the enzyme immunoassay by means of ion selective electrode or an ion sensitive field effect transistor (ISFET), characterized in that at least two enzymes of glucose oxidase and gluconolactonase are used as enzyme-labeled antigens or antibodies.

FIGURE 1A

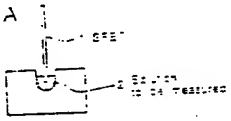


FIGURE 1B

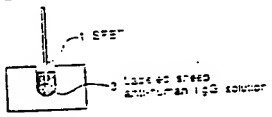
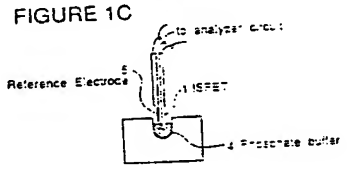


FIGURE 1C



EP 0 328 380 A2

Description

Electrochemical Sensors in Immunological Measurement

Background of the Invention

Field of the Invention

The present invention relates to an electrochemical sensors used in immunological measurement for determining a very small amount of an antigen or an antibody, more particularly it relates to an electrochemical immunochemical sensor system which employes an ion selective electrode or an ion sensitive field effect transistor (ISFET) as a detector electrode in enzyme immunoassay.

Description of the Related Art

Enzyme immunoassay (hereinafter, EIA) which utilizes the specificity of antigen-antibody reaction is one of well-known techniques for determining a very small amount of antigen or antibody. The principle of the EIA is as follow:

In a sandwich assay, an antigen or an antibody to be measured is reacted with a feed antigen or antibody which has a specific reactivity with the antigen or antibody to be measured. After non-reacted antigen or antibody is swept, the bound antigen or antibody is further reacted with enzyme-labeled antigen or antibody which has a specific reactivity or affinity with the antigen or antibody to be measured. After non-reacted antigen or antibody is swept, the proportion of the conjugated enzyme- labeled antigen or antibody is measured by an enzyme reaction, so that the quantity of the antigen or antibody to be measured is determined.

In a competitive assay, a predetermined amount of labeled antigen or labeled antibody is added to a solution containing an antigen or an antibody to be measured. Then, a reaction with a fixed antigen or antibody which has a specific reactivity with the antigen or antibody to be measured is effected. After non-reacted antigen or antibody is swept, the proportion of the conjugated enzyme-labeled antigen or antibody is measured by an enzyme reaction, so that the quantity of the antigen or antibody to be measured is determined.

In the above-mentioned techniques of EIA, the enzyme activity or the proportion of the conjugated enzyme-labeled antigen or antibody can be determined by measuring the absorbance or the fluorescence of a substrate or the enzymic reaction product, by measuring the quantitative change of dissolved oxygen by means of an oxygen electrode or by measuring the pH change by means of an ion selective electrode or the like.

When the enzyme activity is determined by the pH change, an ion sensitive field effect transistor (hereinafter ISFET) can be used as a pH electrode. The ISFET is expected to be a high sensitive sensor or probe for measuring a very small amount of samples precisely in the field of immunological assay.

However, the ion selective electrode or ISFET electrode can not be applied to practical uses in an immunochemical measurment method in which glucose oxidase is used as a labeling enzyme. In fact, when a detector electrode on which an antigen or an antibody labeled with glucose oxidase is attached by an immuno-reaction is immersed in a glucose solution whose enzyme activity is to be determined and whose glucose will be oxidized with glucose oxidase, the pH change does not proceed quickly, in other words the variation in pH value is very small, so that it is difficult to determine the amount of labeling enzyme satisfactorily.

Therefore, an object of the present invention is to overcome the problems in the prior arts and to provide a high-sensitive sensor system which can accelerate the measuring speed and to increase the variation in pH when an ion selective electrode or an ISFET electrode is used as a detector electrode in the enzyme immunoassay.

Summary of the Invention

An electrochemical sensor system used for determining the quantity of an antigen or an antibody by the enzyme immunoassay according to the present invention includes an electrode for detecting the change in electrochemical value and at least one enzyme-labeled antigen or antibody which can be bound to an antigen or antibody to be measure and is characterized in that at least two enzymes of glucose oxidase and gluconolactonase are used as the enzyme-labeled antigen or antibody.

The enzyme immunoassay according to the present invention can be carried out by any one of the conventional techniques including a sandwich assay or a competitive assay.

In the case of sandwich assay or competitive assay, it is used usually a fixed antigen or antibody which has a specific reactivity with the antigen or antibody to be measured in case of the sandwich assay or with the

enzyme-labeled antigen or antibody in the case of the competitive assay.

The electrode can be an ion selective electrode or an ion sensitive field effect transistor (ISFET).

According to a preferred embodiment of the present invention, the antigen or antibody which is labeled with glucose oxidase and gluconolactonase are attached on a surface of a detector electrode by an immuno-reaction and then is immersed in a glucose solution whose enzyme activity is to be determined. In the glucose solution, the following reactions proceed:

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconolactone} + H_2O_2$$

$$\text{gluconolactone} \xrightarrow{\text{gluconolactonase}} \text{gluconic acid}$$

This means that, in the conventional method in which the antigen or antibody is labelled with glucose oxidase alone, it takes a long time until glucose is converted to gluconic acid because glucose is oxidized with glucose oxidase in the first step and then the resulting gluconolactone is slowly hydrolyzed without gluconolactonase into gluconic acid formation of which is detectable by an ion selective electrode or an ISFET. so that the pH change does not occur quickly and hence a big output signal can not be obtained.

To the contrary, according to the present invention in which an antigen or antibody labelled with glucose oxidase and gluconolactonase is used, the gluconolactone which is an oxidation product of glucose is immediately hydrolyzed with gluconolactonase into gluconic acid, so that a big pH change occur and hence a significant output signal which is detectable by the ion selective electrode or ISFET is obtained.

According to the present, higher sensitivity in measurement is assured by the enzyme immuno-sensors which use potentiometric detector electrodes. In fact, when glucose is used as a substrate in the potentiometric enzyme immuno-sensors, if the antigen or antibody labelled with only glucose oxidase as is the conventional amperometric immuno-sensors, a big output signal can not be obtained because of such a fact that glucose is oxidized in the first step with glucose oxidase which is immobilized on a surface of a detector electrode by the immuno-reaction and then it takes a long time until gluconolactone is converted into gluconic acid which is detectable by an ion selective electrode or an ISFET.

To the contrary, according to the present invention, both of glucose oxidase and gluconolactonase are labelled simultaneously. Therefore, in addition to glucose oxidase, gluconolactonase is also immobilized by the immuno-reaction on the surface of the detector electrode and the gluconolactone which is an oxidation product of glucose is immediately hydrolyzed with gluconolactonase into gluconic acid, so that a big pH change occur in a short time and hence the highly sensitive measurement is assured even by the ion selective electrode or ISFET.

Now, an example of the present invention will be described in more details with reference to attached drawings.

Brief Description of the Drawings

Fig. 1A, 1B and 1C illustrate three steps for carrying out the measuring method according to the present invention for determining human IgG by an ISFET on which surface the sheep anti-human IgG is immobilized.

Fig. 2 shows the response characteristics when the ISFET which is subjected to an immuno-reaction is immersed in a glucose solution.

Fig. 3 shows a relation between the output of ISFET and the concentration of human IgG.

Example

In this Example, human immuno globurine G (hereinafter human IgG) is determined.

At first, 5 mg of glucose oxidase and 10 mg of gluconolactonase are dissolved in 0.3 ml of 0.2 M phosphate buffer (pH 6.8) containing 1.25 % of glutaraldehyde and are left at ambient temperature for 20 hours. Then, the solution is applied to Sephadex column equilibrated with 0.15 M sodium chloride (NaCl) and eluted. 1 ml of the resulting eluted solution of glucose oxidase and gluconolactonase is mixed with 1.0 ml of 0.15 M NaCl solution containing 5 mg of sheep anti-human IgG and 0.1 ml of 1 M sodium carbonate buffer (pH 9.5) and is left for 2 hours at 4 °C. The mixed solution is dialyzed against buffered physiological saline to obtain a sheep anti-human IgG labeled with glucose oxidase and gluconolactonase.

By using this sheep anti-human IgG labeled with glucose oxidase and gluconolactonase, the concentration of human IgG is determined as follow:

At first, as is shown in Fig. 1A, an ISFET (1) on a surface of which sheep anti-human IgG is immobilized is immersed in a solution (2) to be measured and containing human IgG for 5 minutes to perform antigen-antibody reaction. Then, the ISFET (1) is immersed in the solution (3) of sheep anti-human IgG labelled with glucose oxidase and gluconolactonase for 5 minutes as is shown in Fig. 1B. After then, as is shown in Fig. 1C, the ISFET (1) is immersed in 0.01 mM phosphate buffer (4) (pH 6.8) the glucose concentration of which is 500 mg/dl to measure the pH change in a membrane fixed on the surface of the ISFET (1).

Fig. 2 shows response characteristics for the respective concentrations of the human IgG of 10 μM, 20 μM and 30 μM, when the ISFET is immersed in the glucose solution after the immuno-reaction. The output signal of ISFET is recorded by a source follower circuit. Fig. 2 reveals such a fact that the output level increase with time elapsed.

Fig. 3 shows a relation between the output of ISFET and the concentration of human IgG in one minute after the ISFET is immersed in the glucose solution.

It is apparent from the Example that a big output signal can be obtained in a short time after the immune-reacted ISFET is immersed in the glucose solution according to the present invention in which both of glucose oxidase and gluconolactonase are used as labeling enzymes, so that higher sensitivity of measurement is assured.

Although glucose oxidase and gluconolactonase are reacted simultaneously with the antibody to obtain the labelled antibody in the Example, it is also possible to react the antigen with glucose oxidase at first and then with gluconolactonase separately.

Claims

1. An electrochemical sensor system used for determining the quantity of an antigen or an antibody by the enzyme immunoassay, including an electrode for detecting the change in electrochemical value and at least one enzyme labeled antigen or antibody which can be bound to an antigen or antibody to be measure, characterized in that said enzyme labeled antigen or antibody is labeled with at least two enzymes of glucose oxidase and gluconolactonase.

2. The electrochemical sensor system set forth in Claim 1, characterized in that the enzyme immunoassay is carried out by a sandwich assay or a competitive assay.

3. The electrochemical sensor system set forth in Claim 1 or 2, characterized by including further a fixed antigen or antibody which has a specific reactivity with the antigen or antibody to be measured in case of the sandwich assay or with the enzyme labeled antigen or antibody in the case of the competitive assay.

4. The electrochemical sensor system set forth in any one of Claim 1 to 3, characterized in that said electrode is an ion selective electrode or an ion sensitive field effect transistor (ISFET).

5. The electrochemical sensor system set forth in any one of Claim 1 to 4, characterized in that said antigen or antibody to be measure is human IgG.

6. Immunological measurment method by enzyme immunoassay for determining the quantity of an antigen or an antibody by an electrochemical sensor, characterized in that at least two enzymes of glucose oxidase and gluconolactonase are used as enzyme labeled antigens or antibodies.

7. The immunological measurment method set forth in Claim 6, characterized in that the enzyme immunoassay is carried out by a sandwich assay or a competitive assay.

8. The immunological measurment method set forth in Claim 6 or 7, characterized in that the antigen or antibody to be measured or the enzyme labeled antigen or antibody is bound to a fixed antigen or antibody which has a specific reactivity them.

9. The immunological measurment method set forth in any one of Claim 6 to 9, characterized in that said electrode is an ion selective electrode or an ion sensitive field effect transistor (ISFET).

10. The immunological measurment method set forth in any one of Claim 6 to 9, characterized in that said antigen or antibody to be measure is human IgG.

FIGURE 1A

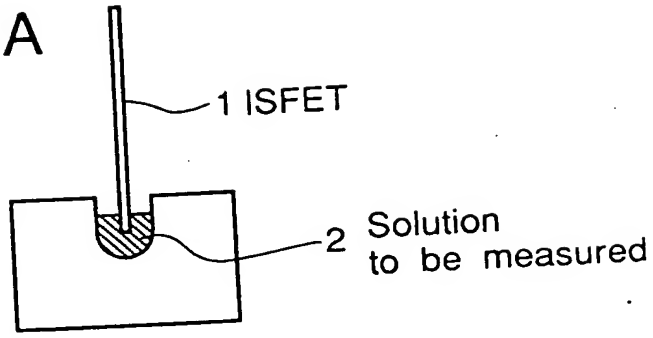


FIGURE 1B

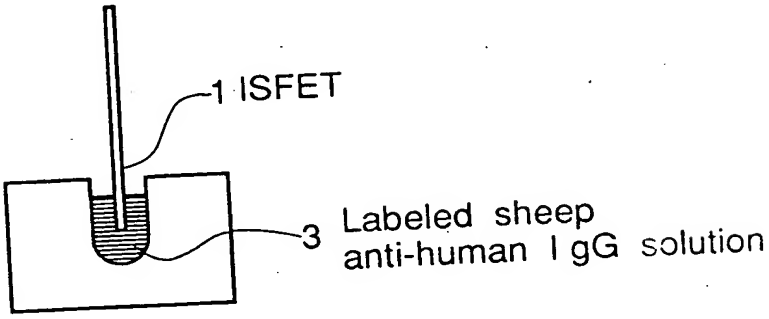


FIGURE 1C

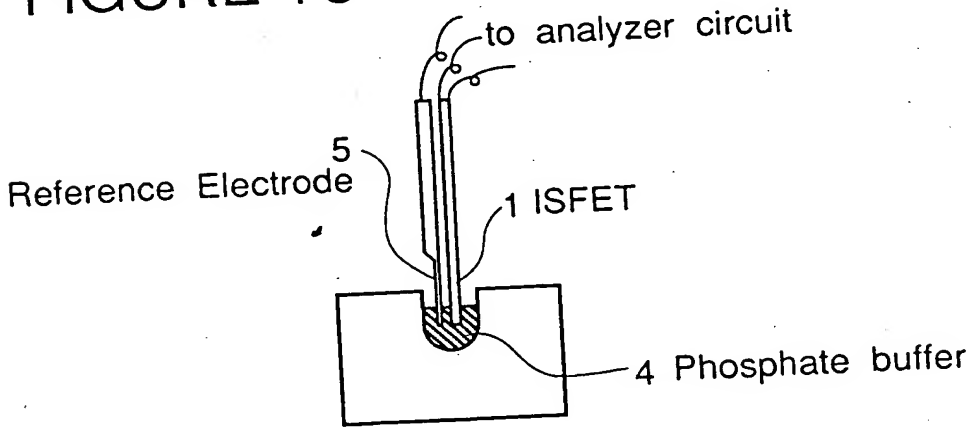


FIGURE 2

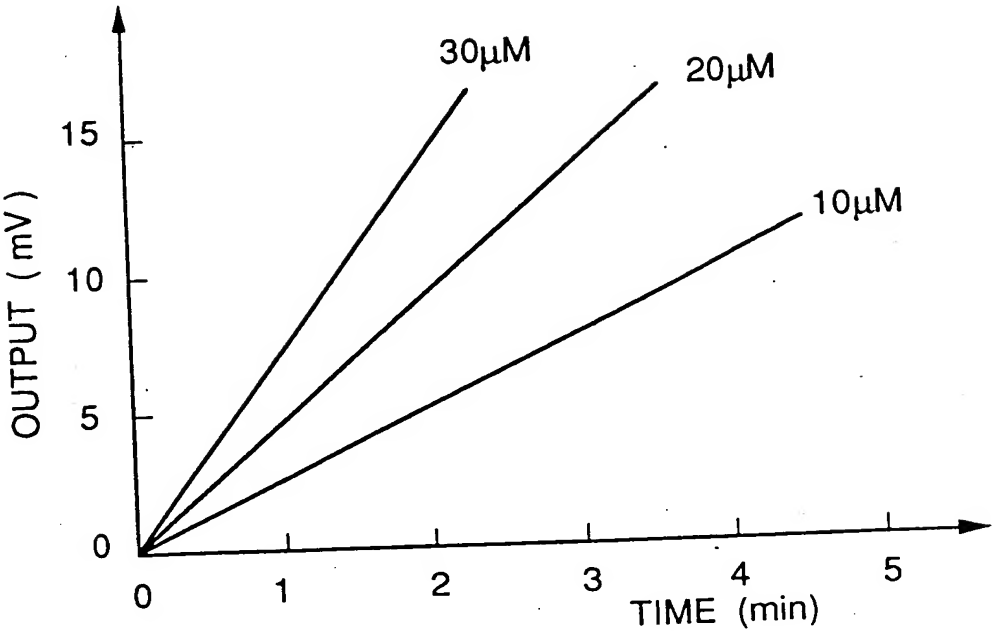


FIGURE 3

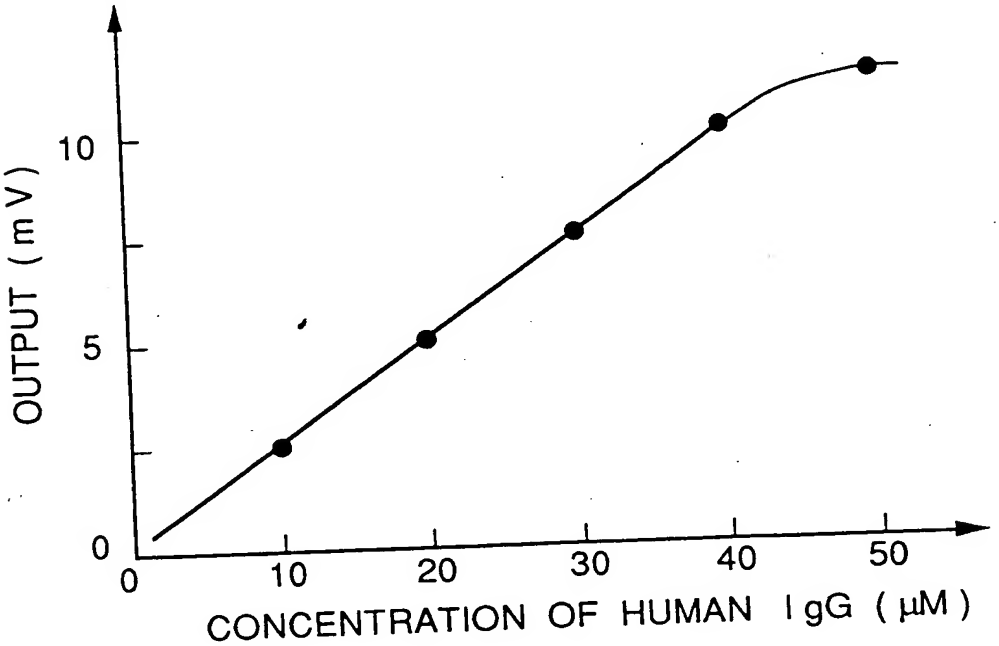


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FIGURE 1A

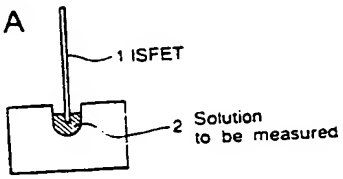


FIGURE 1B

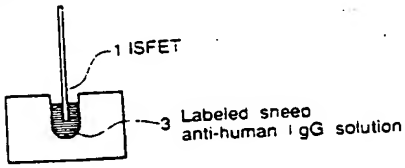
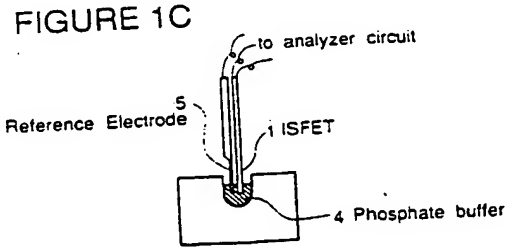


FIGURE 1C



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EUROPEAN SEARCH REPORT

Application Number

EP 89 30 1237

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	EP-A-0 127 438 (NATIONAL RESEARCH DEVOLOPMENT CORP.) * Page 1, line 20 - page 2, line 6; page 3, lines 8-10; page 3, lines 16-18 * ---	1-9	G 01 N 27/00 G 01 N 33/53
Y	CHEMICAL ABSTRACTS, vol. 90, no. 1, 1st January 1979, page 244, abstract no. 2418z, Columbus, Ohio, US; Y.K. CHO et al.: "Immobilization of enzymes on activated carbon: properties of immobilized glucoamylase, glucose oxidase, and gluconolactonase", & BIOTECHNOL. BIOENG. 1978, 20(10), 1651-65 * Abstract, lines 12-16 * ---	1-9	
Y	CHEMICAL ABSTRACTS, vol. 110, no. 19, 8th May 1989, page 667, abstract no. 17859z, Columbus, Ohio, US; H. YOSHIO et al.: "Glucose-sensitive field-effect transistor with a membrane containing coimmobilized gluconolactonase and glucose oxidase", & ANAL. CHIM. ACTA 1988, 212(1-2), 49-59 * Whole abstract * ---	1-10	TECHNICAL FIELDS SEARCHED (Int. Cl.4) G 01 N
Y	EP-A-0 155 193 (SERONO DIAGNOSTICS LTD) * Whole document * -----	1-10	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	04-04-1990	VAN BOHEMEN C.G.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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